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DEVELOPMENT AND BEHAVIOR OF AN INTERGENERIC CHIMERA OF HYDRA (*PELMATOHYDRA OLIGACTIS* INTERSTITIAL CELLS: *HYDRA ATTENUATA* EPITHELIAL CELLS)

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Hydra has so few cell types that it should be possible to map out the developmental and behavioral functions of each. By combining cell types of different species, one might trace roles by identifying species characters in the resulting chimeric animals.

Many of hydra's highly specialized cell types (nerve cells, nematocytes, gametes) are part of a single lineage of cells that is continually being renewed by proliferation and differentiation of a stem cell called the interstitial cell or "I cell." The entire interstitial cell lineage can be removed from a hydra by various means (Marcum and Campbell, 1978a; Campbell, 1979). The resulting animal is termed an "epithelial hydra," and is composed only of ectodermal and endodermal epithelial cells. This viable epithelial shell can then be repopulated by I cells, since they will migrate throughout the depleted animal from a small, temporary graft of normal tissue. A number of chimeric strains in hydra have been made in this fashion (Saffitz, Burnett and Lesh, 1972; Sugiyama and Fujisawa, 1978; Marcum and Campbell, 1978b). In order to assign roles to the different cell lineages, one would use the most dissimilar species as parents. However, grafting success (and hence presumably tissue compatibility) decreases as species diversity increases (Campbell and Bibb, 1970), so that most hydra chimeras have been constructed of cells from the same or closely related species.

One pair of dissimilar species, *Hydra attenuata* and *Pelmatohydra oligactis*, will partially tolerate intergrafting and a considerable literature suggests that it may be possible to make a stable chimera between their cells (Evlakowa, 1946; Brien and Reniers-Decoen, 1955; Kolenkine and Bonnefoy, 1976). Since it is possible to remove the interstitial cell lineage from *H. attenuata*, we repopulated epithelial *H. attenuata* with *P. oligactis* interstitial cells. The reciprocal graft is not possible since a technique for removing I cells from *P. oligactis* has not been found. This report describes some developmental and behavioral similarities and differences between the chimeras and the two parental species.

Material and Methods

Specimens of *Hydra attenuata* from Lake Zurich, specimens of *Pelmatohydra oligactis* collected in Grant Lake, Mono County, California, and the chimeras were all grown in "M" solution lacking bicarbonate (Muscatine and Lenhoff, 1965) by standard methods (Lenhoff and Brown, 1970). Epithelial specimens of *H. attenuata* were produced by a double colchicine treatment (Marcum and Campbell,

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1978a) to eliminate I cells. Distal halves of epithelial specimens of *H. attenuata* were repopulated with I cells by axially grafting them to proximal halves of normal *P. oligactis* polyps. The graft junction was marked by a permanent constriction in the ectoderm (Kolenkine and Bonnefoy, 1976) and by different coloration of the endoderm in these two species. Grafts were left intact for 3 to 5 days and then the *P. oligactis* epithelial tissue was cut away. The resulting repopulated hydra were maintained for up to 6 months by methods appropriate for epithelial hydra (Marcum and Campbell, 1978a).

Cell compositions were determined using David's (1973) maceration procedure. Heat shocks were applied by immersing 10-ml test tubes containing individual hydra in 2 ml of medium into a preheated waterbath for 30 min. Afterwards the tubes were left at room temperature for 12 hr, and then the hydra were cultured normally. Time-lapse films were made using a 16-mm Bolex camera outfitted with extension tubes behind the lens, with illumination through heat filters.

Feeding response was measured using the methods of Lenhoff (1969). Inhibition of nematocyst discharge (Smith, Oshida and Bode, 1974) was carried out by feeding hydra to repletion and then releasing single shrimp, successively, onto tentacles at measured times later. The number of shrimp contacts which occurred before two became trapped was recorded. Only 20 trials were offered each polyp; a score of 20 indicated that the hydra did not catch the *Artemia*.

Results

Genetic composition of chimeras

Although the methods used for producing chimeras seem straightforward, we considered it important to demonstrate that the chimeras were, in fact, composed of *H. attenuata* epithelial and *P. oligactis* interstitial cells.

The genetic origin of interstitial cells was verified by analysis of chimera nematocysts. Table I shows the dimensions and Figure 1 shows the morphologies

Table I

Nematocyst sizes. Length and width (standard deviations in parenthesis) are all in μm. Each measurement represents about 20 nematocysts. Chimera polyps a, b, c, and d are progeny from different grafts.

Hydra strain	Stenotele	Atrichous isorhiza	Desmoneme
H. attenuata	14.8 × 11.4 (2.2) (1.9)	9.7 × 4.0 (1.0) (0.2)	8.1 × 5.6 (0.6) (0.4)
P. oligactis	12.9 × 9.7 (0.5) (0.5)	7.8 × 4.3 (0.5) (0.5)	6.7 × 4.8 (0.4) (0.3)
Chimera polyp a	12.1 × 9.5 (0.7) (0.7)	8.2 × 4.0 (0.7) (0.2)	5.7 × 4.2 (0.5) (0.4)
polyp b	11.9 × 9.1 (0.6) (0.8)	8.8 × 4.4 (0.5) (0.6)	6.2 × 4.6 (0.4) (0.4)
polyp c	11.0 × 8.3 (1.0) (0.7)	8.8 × 3.8 (0.6) (0.3)	6.1 × 4.0 (0.5) (0.1)
polyp d	11.7 × 8.9 (0.6) (0.5)	9.1 × 4.0 (0.8) (0)	6.1 × 4.2 (0.2) (0.2)

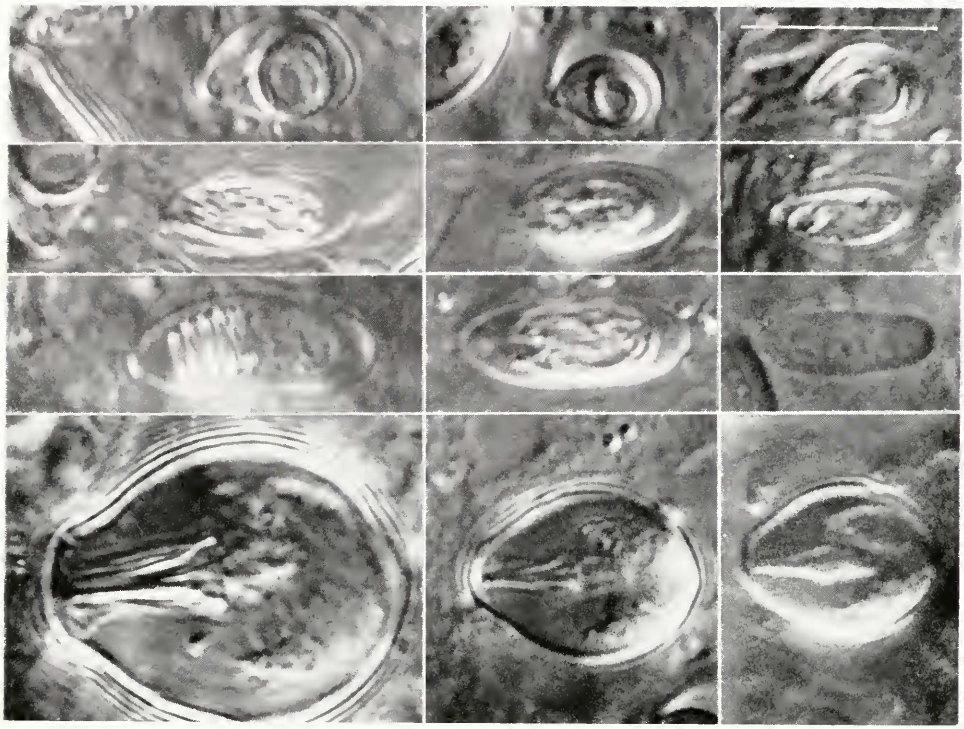

FIGURE 1. Nematocyst structures of *H. attenuata* (left), *P. oligactis* (middle), and chimera (right). Nematocyst types, bottom to top, are: stenotele, holotrichous isorhiza, atrichous isorhiza, and desmoneme. All figures except the chimera holotrichous isorhiza represent mature, mounted nematocysts. Chimeras rarely have mounted holotrichous isorhizas, but the species specific tubule pattern is visible in complete but immature capsules in gastric region nests, as shown here. Scale indicates 10 μm.

of nematocysts of the chimeras and both parents. These data are taken from animals more than 4 months after chimeras were made. The small sizes of the chimera nematocysts are similar to those of *P. oligactis* and not *H. attenuata*. The morphologies of the chimera nematocysts (Fig. 1) are also unambiguously those of *P. oligactis*. The absence of transverse coils in the holotrichous isorhiza is the

TABLE II

Relative proportions of nematocyst types in tentacles. Numbers express percentages of total nematocysts. (Between 500 and 1000 nematocysts were counted in the distal parts of tentacles, with the number of polyps indicated at left).

Species	Nematocyst type		
	Stenotele	Isorhizas	Desmoneme
H. attenuata (n = 2)	3.5 (±0.7)	12.5 (±4.9)	84 (±4)
P. oligactis (n = 3)	34 (±9)	5.7 (±1.5)	60 (±9)
Chimera (n = 6)	37 (±8)	6.7 (±1.0)	57 (±7)

most notable character of *P. oligactis* stingers (Ewer, 1948), and the chimera also lacks these. In addition, the slender shape of the stenotele, the bluntly oval shape of the holotrichous isorhiza, and the reniform atrichous isorhiza are all characteristically *P. oligactis*

The two parental species differ in the relative abundances of the different nematocyst types. Table II shows that in this character the chimeras closely resemble *P. oligactis* and not *H. attenuata*. However, one abnormality of the chimeras was the nearly complete absence of mature holotrichous isorhizas. None were found on animals whose nematocytes were measured (Table I), and few were seen in this study. The photograph in Figure 1 is of an immature holotrichous isorhiza.

The genetic origin of epithelial cells was ascertained in two ways. First, in color both the ectoderm and endoderm were found to resemble *H. attenuata* rather than *P. oligactis*. The chimeric ectoderm was colorless, and the endoderm pink, as in *H. attenuata*. *P. oligactis* has yellow granules in the ectoderm and an orange endoderm. Second, it is known that epithelial tissue controls graft tolerance (Campbell and Bibb, 1970). Therefore, two chimeras that had been established for 6 months were bisected and halves were grafted back to the two parental strains. In the chimera/*H. attenuata* grafts, the graft junctions became imperceptible within a day and no incompatibility was detected during the next 8 days of culture. In the chimera/*P. oligactis* grafts, the graft junctions were still constricted after 1 day and by the sixth day the two halves had separated.

We conclude that the chimeras had *H. attenuata* epithelial cells and *P. oligactis* interstitial cell lineage, and that this composition remained stable throughout the study.

Morphology of chimeras

Chimeras were always smaller than either parent (Fig. 2). Measurements of ten chimeras and parents grown on a regime of six shrimps/day averaged 2.0 mm (chimera), 7.0 mm (*H. attenuata*) and 9.0 mm (*P. oligactis*) in extended length. Tentacle number of budding individuals averaged 5.8 (chimeras), 6.5 (*H. attenuata*) and 6.4 (*P. oligactis*) per polyp. The body column and tentacles of chimeras never seemed to elongate as much as those of either parental species, and this was a contributing factor to their short lengths. The chimeras were slightly more stalked than the *H. attenuata* parent, but not as pronouncedly so as the *P. oligactis* parent.

Budding

The most clear-cut difference between buds of the parental species is the arrangement of tentacle rudiments. Buds of *P. oligactis* first acquire two lateral tentacles, and after these have grown long, two more intercalated rudiments arise (Fig. 3a). In *H. attenuata* the tentacle rudiments arise nearly synchronously and are all about the same length (Fig. 3c). The chimeric pattern (Fig. 3b) is clearly of the *H. attenuata* type.

The budding rates of chimeras were about normal. In one experiment three polyps each of *H. attenuata*, *P. oligactis* and chimera were fed six shrimps/day,

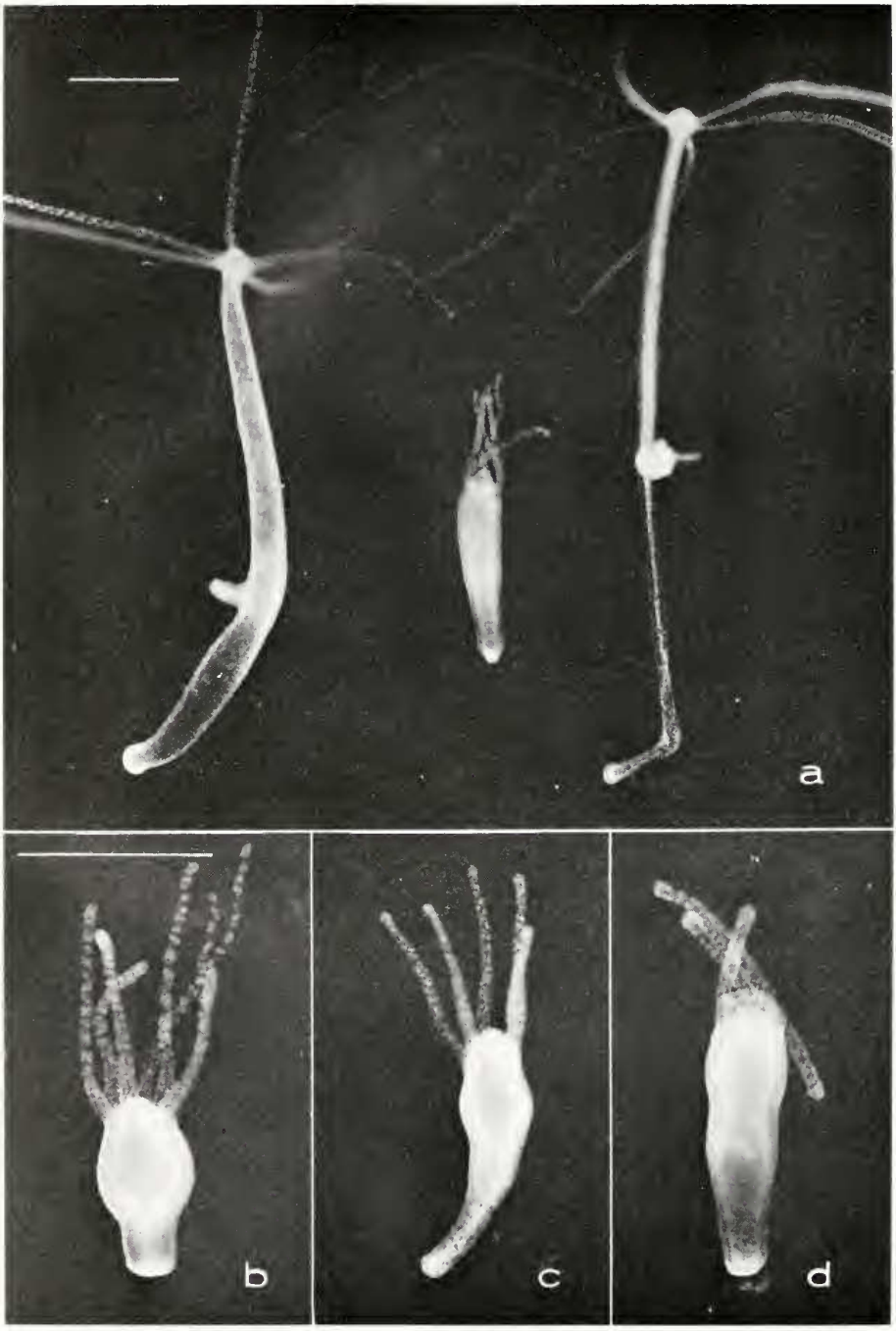

FIGURE 2. Morphology of hydra. (a) *H. attenuata* (left), chimera (middle) and *P. oligactis* (right). All three hydras are of the same age, growing under the same conditions. (b)–(d) different chimera individuals showing typical poses. Scale indicates 1 mm.

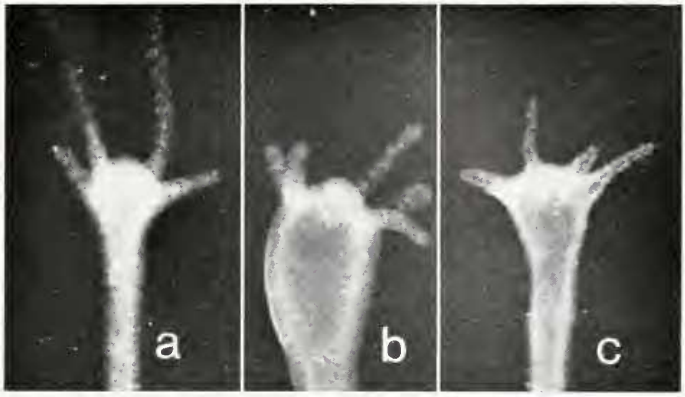

FIGURE 3. Pattern of tentacle development on buds. (a) *P. oligactis* (b) chimera, (c) *H. attenuata.*

long enough for each to produce five buds. Budding rates for these three strains were, respectively, 0.26, 0.26, and 0.20 buds/day. Early morphogenesis, until basal disk formation, of the developing buds occurred at normal rates. However, chimera buds remained attached to their parents for an average of 8 days, while both parental buds detached after 3 days. Mature chimera buds were very small, averaging 1.2 mm in length (extended), whereas *H. attenuata* buds average 5.1 mm and *P. oligactis* buds averaging 5.4 mm in length. Chimera buds did not themselves begin to bud for 19 days, while parental buds budded after 10 (*H. attenuata*) or 11 (*P. oligactis*) days.

Regeneration

Polyps that had been fed six shrimps/day for 6 days were cut in half transversely and both parts allowed to regenerate, without feeding. Tentacle regeneration was assayed by the presence of tentacle rudiments. Basal disk regeneration was assayed by adherence to the dish.

The chimeras ($n = 3$) and both parental species (*H. attenuata,* $n = 8$; *P. oligactis,* $n = 7$) regenerated tentacles from the proximal half in 2 days. The distal halves of chimeras and of *H. attenuata* regenerated basal disks in 2 days. However, the *P. oligactis* proximal halves had not regenerated basal disks in 26 days. Thus, in basal disk regeneration the chimera resembled the epithelial cell parent.

Feeding behavior

The chimeras (as well as the two parental strains) showed typical (Lenhoff, 1969) feeding responses to *Artemia.* When shrimp touched the tentacles they adhered, indicating desmoneme nematocyte discharge, and were paralyzed, indicating stenotele nematocyte discharge. Tentacles holding shrimp underwent considerable writhing, and shrimp were brought to the mouth repeatedly. However, in the chimera the shrimp were never swallowed. The chimeras were thus unable to feed themselves.

To determine if the swallowing behavior itself was deficient in the chimeras,

TABLE III

Inhibition of nematocyst discharge following satiation. Numbers represent average number of trials before hydra caught and paralyzed two Artemia. *Twenty was the maximum number of trials allowed for any polyp. These data represent about 100 sets of trials.*

Time after feeding (min.)	Hydra strain		
	H. attenuata	*P. oligactis*	Chimera
10–30	5.8	7.5	5.4
30–60	12	16	8.4
60–120	10	20	6.7

we strung isolated hydranths on nylon fishing line in "M" solution and measured the creeping movement along the line. In normal hydra the hydranth will move rapidly along the line in the direction of the mouth, as the hydranth attempts to swallow the line. After the first 20 min, *H. attenuata* ($n = 1$) had moved 2.4 mm and *P. oligactis* ($n = 1$) had moved 0.9 mm. Two chimera hydranths failed to move during 3.5 hr of observation.

Normal hydra exhibit a mouth opening response to glutathione. The duration of opening reflects the intensity of the response (Lenhoff, 1969). The durations of the feeding responses to 10^{-5} M glutathione (26° C, pH 7.0) were measured with the following results: *H. attenuata* ($n = 10$), 35.2 ± 18.6 min; *P. oligactis* ($n = 11$), 41.9 ± 13.5 min; chimera ($n = 26$), 21.7 ± 6.8 min.

We also tested the satiation response, as manifested by the failure of nematocysts to discharge, and consequently the failure to trap *Artemia*, after the hydra is fed (Smith, Oshida and Bode, 1974). Table III shows data from these experiments. The chimeras showed a reduced satiation response up to two hours after feeding.

Body motility

Chimeras were much less active than were the two parents. Chimeras never somersaulted nor extended fully, while parental polyps frequently did. We analyzed body contractions and pulsations using time-lapse motion pictures showing all three polyp types in the same dish. By "contractions" we refer to a marked shortening of the body column followed by a reextension. "Pulsation" refers to peristaltic waves traveling proximally down the column.

In contraction frequency, chimeras (0.25/min) were intermediate between *H. attenuata* (0.08/min) and *P. oligactis* (0.34/min). In pulsation frequency chimeras (1.07/min) resembled *H. attenuata* (1.03/min). *P. oligactis* did not exhibit pulsations.

DISCUSSION

The major objective of this research was to produce a chimeric strain of hydra in order to distinguish developmental and behavioral contributions of the epithelial and interstitial cell lineages. Other studies of this nature (Sugiyama and Fujisawa, 1978; Marcum and Campbell, 1978b) showed the feasibility of this approach using closely related strains or species. In the present study we sought to examine the

feasibility of using distantly related species, in this case species of separate genera.

In several traits it was possible to attribute chimeric development and behavior to particular cell types. The pattern of tentacle origin on buds, the rate of basal disk regeneration, heterospecific grafting specificity, color, and prominence of columnar peristaltic waves were all clearly characteristic of *H. attenuata.* Thus, these traits are determined by epithelial cells. On the other hand, nematocyst morphology, nematocyst concentration, and interstitial cell temperature sensitivity (Fradkin, Lee, and Campbell, unpublished) were distinctively those of the *P. oligactis,* indicating that these characteristics are due to the interstitial cell lineage. These results fit the pattern so far uncovered (Campbell, 1979) that morphological and morphogenetic traits derive principally from the epithelial cell genotype.

In several respects the evolutionary divergence between *H. attenuata* and *P. oligactis* was apparently too great to allow normal chimera functioning. These chimeras were very delicate, and had such altered behavior that they could neither eat nor sommersault by themselves. Repopulation of epithelial hydra by interstitial cells of the same species yields hydra of normal behavior (Marcum and Campbell, 1978b) suggesting that the behavioral defects observed here are due to cell incompatibilities rather than due to the repopulation procedure. Therefore, in constructing chimeras for purposes of deducing cellular roles one must work with more closely related species. However, chimeras containing cells as divergent as *H. attenuata* and *P. oligactis* certainly may be useful in unravelling cellular mechanisms. It would be interesting, for example, to see if abnormal neuromuscular contacts might be responsible for the inadequacy of the feeding behavior or the absence of column elongation.

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Summary

An intergeneric chimera was produced by repopulating epithelial *Hydra attenuata* (lacking the interstitial cell lineage) with interstitial cells of *Pelmatohydra oligactis.* The chimera's morphology and morphogenesis generally resembled that of *H. attenuata,* for example in the pattern of bud tentacles, in basal disk regeneration rate, and in heterografting specificity. Nematocyst characters of the chimera were the *P. oligactis* type. In behavior the chimeras were intermediate in some respects but deficient in others. For example, chimeras were unable to feed by themselves or to extend the column. This study illustrates the value of chimeras in deducing which cell types control the various aspects of development and behavior.

Note added in proof

Data from cell type composition studies show that the percentage of nerves among the total cells counted for *H. attenuata* is 5.5%, for *P. oligactis,* 5.7%, and for the Chimera, 6.4%. A total of 5000 to 9000 cells were counted for 6 to 9 individuals (6 individuals of *H. attenuata,* 6 of *P. oligactis,* and 9 of Chimera).

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